bio-protocol Preprint

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Isolation and cell culture of primary alveolar macrophages

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An abbreviated version of this protocol was published in eLIFE in Nov 2018

Selective agonist of TRPML2 reveals direct role in chemokine release from innate immune cells

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Detailed protocol

Required equipment

- Dissection tools (all sterilized with 70% EtOH)
  - 1 x Larger scissors for (dissection)
  - 1 x Smaller scissors (puncturing trachea)
  - 1 x Blunt forceps (dissection)
  - 2 x Sharp forceps (dissection)
  - 1 x Dissection board (styrofoam lid, covered with aluminium foil)
  - 1 x Dissection space, ideally a laminar flow hood (sterilized with 70% EtOH)
- Reagents (for one isolation)
  - 7 mL Sterile magnesium/Calcium-free PBS (DPBS, Gibco, cat. no. #14190-094)
  - ~5 mL RPMI1640 medium (Gibco, cat. no. 61870036), supplemented with 10% FBS (FBS Superior, Biochrom)
- Material
  - 7 x Needles (for fixation) - One needle being long and sturdy for fixing head
  - Suture material
  - 1 x Catheter, sterile (Introcan 1.10 x 32mm G20, #4252110B, Braun)
  - 8 x 1-mL Syringes, sterile (Braun)
  - Kleenex tissue
  - 1 x 15 mL Falcon tube, sterile, on ice

Protocol

image.png

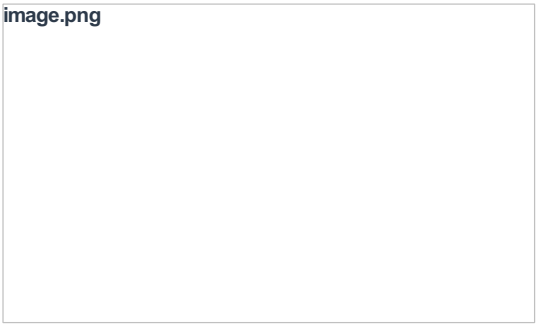

Figure: The exposed trachea (step 7)

1. Euthanize the mouse upon injecting lethal narcosis intraperitoneally
   a. Note: Cervical dislocation and decapitation are unsuitable forms of euthanisia, as the trachea must be kept intact. It is unclear whether inhalative forms of euthanasia (CO_2, isoflurane) impairs macrophage viability
2. Upon loss of the paw withdrawal reflex, fix the mouse by each of it limbs
3. Partially open the peritoneum, and terminally euthanize the mouse by cutting the femoral artery. Soak up blood using the Kleenex tissue, and fix the tissue using two needles.
   a. Note: While being a legally required step to confirm euthanasia, cessation of circulation also reduces the risk of red blood cells contaminating the primary macrophage culture
4. Expose the diaphragm by completely opening the peritoneum, pulling out the intestines and liver
   a. Note: When removing the liver, also separating the gallbladder by cutting the bile duct can ease the exposure of the diaphragm
5. Use a pair of scissors to puncture the diaphragm, then open the scissors to increase the hole size. This should ease resistance upon the lungs, allowing

5. Use a pair of scissors to puncture the diaphragm, then open the scissors to increase the hole size. This should ease resistance upon the lungs, allowing injection of PBS at point .
6. Secure the head firmly by placing the larger needle through the inside of the jaw, immediately next to the trachea
    a. Note: Take care not to damage the trachea by placing the needle slightly to its side and angling it inwards.
7. Expose the thorax by cutting along the ribcage, and continue cutting through the skin along the neck until reaching the jaw, as indiciated in the figure above
    a. Note: Do not puncture the jugular vein at any stage of the isolation protocol, as this could contaminate the primary culture with red blood cells
8. Using the two sharp forceps, remove the glands and layers of skin surrounding the trachea
9. Upon exposing the trachea (it should be clearly visible as a tube lined by bony rings), use the sharp forceps to losely tie a surgical suture around it
    a. Note: Keep the sharp forceps angled slightly away from the trachea to avoid puncturing it
10. Using the smaller, sterile set of scissors, make a partial incision into the trachea, leaving a hole barely large enough to fit the catheter
11. Cannulate the trachea by inserting the catheter
    a. Note: Pull back the catheter needle slightly upon inserting it to avoid it puncturing the trachea. Keeping the needle partially inside the catheter provides stability when inserting it, easing the cannulation. While pushing the catheter deeper into the trachea and lungs, simultaneously slide the needle back until it is completely removed.
12. Tie the surgical suture around the catheter to firmly fix it into place
13. Fill 8 1-mL syringes with DPBS, loading each with 800 uL DPBS and avoiding air bubbles
14. Gently infuse the fluid from the first syringe into the lung. The chest should rise as the DPBS is infused.
15. Massage the thorax for about 10 seconds
16. Crucial step: Gently withdraw the DPBS from the lung. Should any red liquid be withdrawn, immediately remove the syringe and discard the mouse. If the blood contamination does not reach the syringe, the DPBS can be used to isolate alveolar macrophages.
    a. Note 1: Withdrawing the DPBS too quickly often leads to the lung epithelium rupturing, and blood contaminating the culture. Should the resistance become too substantial, rather re-infuse a little DPBS, massage the thorax, and try withdrawing the DPBS again.
    b. Note 2: Particularly upon the first lavages, the return will be much smaller than the infused 800 uL. Do not try salvaging all of the liquid, rather settle for recovering (at least) 0.4 mL at the early flushes. Trying too hard to get more cells in early flushes can compromise the lung integrity, and reduce the number of lavages you can perform. During later flushes, the return should approximate the volume infused.
    c. Note 3: The first flush will also contain chemokines natively secreted into the lung. The first flush can thus be stored and used for e.g. ELISAs.
    d. Note 4: The first flushes contain the most cells, later flushes contain less cells. It is more important to keep the first flushes clean and discard later flushes, should there be reason to suspect blood contamination.
17. Remove syringe from catheter, and gently inject withdrawn DPBS into the 15 mL falcon tube on ice.
18. Repeat steps 14-17 with each of the 8 syringes.
19. Centrifuge the 15 mL falcon tube (400 g, 10 minutes, 4oC) to pellet cells
20. Transfer 15 mL falcon tube to cell culture, and discard supernatant
    a. Note: Collect supernatant if you are profiling chemokines from the early fractions
21. Resuspend pellet in 1 mL RPMI1640 medium, and count the cells
22. Seed cells at an appropriate density on poly-L-lysine-coated glass coverslips
23. Alveolar macrophages do not proliferate well, so consider using the cells for experiments within the next 2-3 days

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Krogsaeter, E. K., Spix, B. , Butz, E. , Rosato, A. S. and Grimm, C. (2020). Isolation and cell culture of primary alveolar macrophages. Bio-protocol Preprint. bio-protocol.org/prep251.
2. Plesch, E., Chen, C., Butz, E., Scotto Rosato, A., Krogsaeter, E. K., Yinan, H., Bartel, K., Keller, M., Robaa, D., Teupser, D., Holdt, L. M., Vollmar, A. M., Sippl, W., Puertollano, R., Medina, D., Biel, M., Wahl-Schott, C., Bracher, F. and Grimm, C.(2018). Selective agonist of TRPML2 reveals direct role in chemokine release from innate immune cells. eLIFE. DOI: 10.7554/eLife.39720